Signature: Med Sci Monit, 2003; 9(2): CR67-72
PMID: 12601289

Received: 2002.12.03
Accepted: 2002.12.06
Published: 2003.02.25

Impact of *Chlamydia pneumoniae* infection on survival rate after heart transplantation

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Piotr Pieniążek[1], Ewa Stępień[2], Jerzy Sadowski[2], Tadeusz Przewłocki[1], Andrzej Sokołowski[3], Piotr Podolec[1], Bogusław Kapelak[2], Piotr Przybyłowski[2], Andrzej Kądzielski[4], Wiesława Tracz[1]

[1] Department of Cardiac and Vascular Disease, Institute of Cardiology, Jagiellonian University School of Medicine, Cracow, Poland
[2] Department of Cardiovascular Surgery and Transplantology, Institute of Cardiology, Jagiellonian University School of Medicine, Cracow, Poland
[3] Department of Statistics, Cracow University of Economics, Poland
[4] Department of Hemodynamics and Angiocardiography, Institute of Cardiology, Jagiellonian University School of Medicine, Cracow, Poland

Summary

Background: As *Chlamydia pneumoniae* (Cp), a common cause of respiratory infection, is of vasotropic character, chronic infection may be associated with the development of coronary disease, although there have been few reports on the impact of Cp infection on the post-orthotopic heart transplantation (OHT) survival rate.

Material/Methods: A total of 41 patients (4 females) were followed up for one year after OHT. Serology investigations for IgM, IgG and IgA antibodies against Cp were performed using the enzyme immunoassay (EIA) method. Univariate and multivariate analyses were carried out with respect to IgA, IgG, gender and type of cardiomyopathy. The IgA-IgG joint effect was also studied.

Results: The one-year survival rate was reported for patients with IgA < 8 EIU to be 72.2%, whereas those with IgA ⩾ 8 EIU accounted for only 43.5% (Kaplan-Meier analysis, p = 0.0548). In multivariate analysis IgA /IgG status proved to be a highly significant factor in survival. IgA positive outcome combined with IgG negative outcome showed that the relative risk of death equaled 12.08 versus other combinations of IgA/IgG status. In the Cox multivariate model ischemic cardiomyopathy showed a relative risk of 2.79 (p=0.0594), although it was not significant in univariate analysis.

Conclusions: Chronic Cp infection, as expressed through a high IgA level, seems to have adverse impact on the survival rate in one-year follow-up after OHT. IgA titers against Cp in heart transplant recipients should therefore be assessed, as the high values might be a predictive risk factor within the first post-operative year.

key words: ***Chlamydia pneumoniae* infection • ischemic cardiomyopathy • orthotopic heart transplantation • survival rate**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_9/no_2/3324.pdf
Word count: 2423
Tables: 1
Figures: 3
References: 35

Author's address: Piotr Pieniążek, MD, PhD, Department of Cardiac and Vascular Disease, Institute of Cardiology, Jagiellonian University School of Medicine, ul. Prądnicka 80, 31-202 Kraków, Poland, email: kardio@kki.krakow.pl

BACKGROUND

Infectious complications and early graft failure (EGF) are widely believed to be one of the principal causes of cardiac death after orthotopic heart transplantation (OHT) within the peri-operative period [1,2]. Within the first three months following surgery, infections are the key morbidity factors, accounting for 75% of all fatalities [1]. According to Kirsch et al. [1], morbidity within the peri-operative period caused by various infections accounts for 30% of all deaths. Similarly, a high morbidity rate due to early graft failure has also been observed within the same period, including unexplained acute early graft failure, pulmonary hypertension, right ventricle failure and hyperacute rejection.

In long-term OHT follow-up, such etiological factors as opportunistic microbes of *Toxoplasma gondi, Candida albicans, Cytomegalovirus, Pneumocistis carini, or Herpes simplex* are reported to cause chronic infections [2]. Grattana *et al*. [3] found that cytomegalovirus infection may effectively exacerbate transplant rejection processes, as well as accelerate atherosclerosis in the implanted heart. Initial reports by Saikku et al. [4,5], later corroborated by other authors [6,7,8], indicate that *Chlamydia pneumoniae* (Cp) infection may significantly accelerate the progress of atherosclerosis and coronary artery disease.

Cp, an intracellular gram-negative bacterium, causes infections of the respiratory system, frequently of chronic and asymptomatic character. It may also invade the circulatory system, causing inflammation in the arterial walls, and by the same token exacerbating or accelerating atherosclerosis [9].

There have been to date very few reports dealing with the incidence of Cp infections in transplant recipients and its impact on prognosis in short- and long-term follow-up after OHT. It is widely acknowledged that ischemic cardiomyopathy is the most common reason for referring patients with severe heart failure for OHT. Chronic Cp infection is known to have a significant bearing on the development of cardiac allograft vasculopathy (CAV), which in due course accounts for much worse long-term prognosis after heart transplantation [10].

No studies have established to date whether there may be any correlations between outcome and past, chronic and acute Cp infections, as measured by anti-Cp IgG, IgA and IgM antibodies in the transplant recipient during OHT respectively, which could be of use as prognostic factors in the postoperative follow-up. The present study is therefore aimed at addressing this issue.

MATERIAL AND METHODS

The present study involved 41 consecutive patients (including 4 females), mean age 50 years (range 28–68) subjected to OHT in the period from July 1998 to March 2000. The patients were referred for OHT on the basis of comprehensive clinical investigations (including coronary angiography, ventriculography, right ventricle heart catheterization, and pulmonary resistance, as well as endomyocardial biopsy). The biopsy, however, was not performed in patients diagnosed with diffuse coronary atherosclerosis combined with severe left ventricular impairment. In all the patients included in the study, the peak oxygen consumption was below 10 ml/kg/min, which has been shown to be associated with the worst prognosis [11].

The patients subjected to OHT fell well within the criteria for NYHA functional class IV, since they were diagnosed with progressive heart failure, despite having been on multi-directional pharmacotherapy. Seventeen patients were subjected to heart transplantation due to idiopathic cardiomyopathy, whereas 24 patients were qualified for surgery due to ischemic cardiomyopathy.

Immunosuppression treatment consisted of induction therapy using anti-thymocyte globulin (ATG) administrated at a dose of 2.5 mg/kg/day, commencing immediately after surgery and continuing for 4–6 days. Additionally, patients were treated with triple therapy based on cyclosporin, azathioprin or mycophenolate mofetil, and steroids.

Endomyocardial biopsy specimens were obtained with a view to monitoring any possible graft rejection process on routine protocol. Serological testing for Cp was carried out within the first 24 hours of OHT.

In order to assess prior and current *Chlamydia pneumoniae* infection serologically, IgG, IgA, and IgM levels in the plasma of patients after heart transplantation were determined using a single-point serology method. Plasma was collected and stored at –26°C for up to two weeks prior to the examination. The levels of IgG, IgA and IgM antibodies were determined using commercial EIA tests, in compliance with the manufacturer's instructions (Labsystem). The results were interpreted in line with the criteria established by the manufacturer. An increased IgG level (⩾30 EIU) indicates past or current Chlamydia infection. A persistent high level of IgG antibodies encountered in an immunocompetent subject makes it possible to detect IgG even two years after infection. Primary infection is characterized by an increased level of IgM only. An increased IgM titer may still be observed within 2–4 weeks after primary infection and is subsequently followed by IgG response. IgM antibodies are usually undetectable 2–6 months after primary infection.

An elevated or high IgG level, combined with persistent, short-lived IgA, is considered to be a marker of chronic Cp infection. In order to maintain consistency of interpretation between EIA and the widely used microimmunofluorescence methods, all samples equal to, or in excess of, 8 EIU, were considered positive.

Statistical analysis

Survival curves were estimated by Kaplan-Meier method and then compared with the log-rank test. Multivariate analysis was performed using a Cox model.

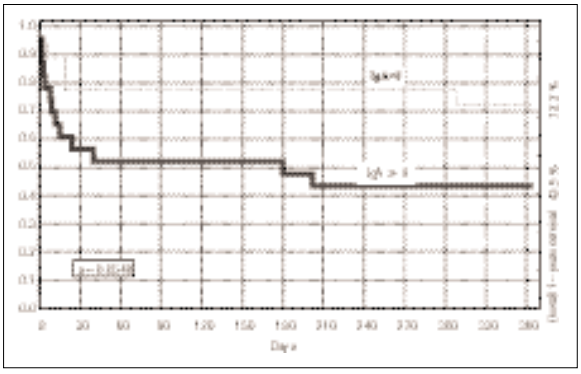

Figure 1. Kaplan-Meier survival curves related to IgA level.

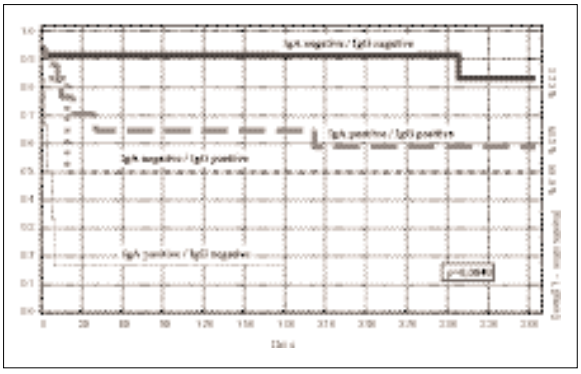

Figure 2. Kaplan-Meier survival curves for IgA/IgG combinations.

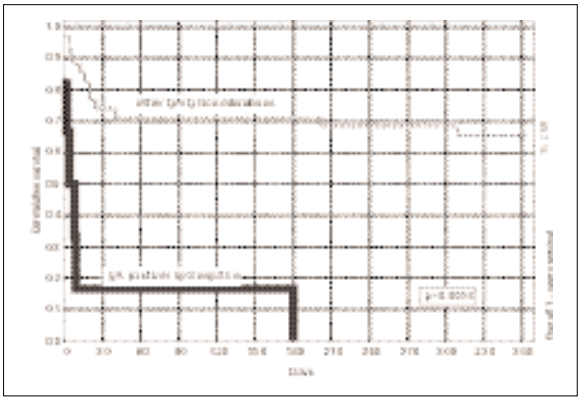

Figure 3. Kaplan-Meier survival curves related to IgA positive/IgG negative versus other IgA/IgG combinations.

The final model was obtained through the stepwise regression approach. All the calculations were performed on STATISTICA v. 6.0 software.

Results

Out of the 41 patients after heart transplantation, 18 (43.9%) died within the first 360 days of the follow-up period (the mean age of the deceased was 54 years, whereas the mean age of the survivors was 48 years). Of the 18 deceased, 7 (38.9%) died due to early graft failure, 5 (27.8%) due to multi-organ insufficiency, principally of cardio-pulmonary character, 4 (22.2%) due to infections, and 2 (11.1%) as a result of transplant rejection process.

Table 1. Cox proportional hazard model.

Variable	Value	n	Relative risk	p-value
IgA/IgG status	IgA positive/ IgG negative	6	12.08	0.0000
	Other combinations	35	1.00	–
Cardiomyopathy	Idiopathic	17	1.00	–
	Ischemic	24	2.79	0.0594

No increased level of IgM antibodies was encountered in any of the 41 patients, which ruled out the possibility of acute Cp infection at the time of OHT. Elevated titers of IgG were encountered in 23 patients (56.1%), whereas elevated IgA was also found in 23 patients (56.1%).

In our univariate analysis, the survival curves were compared by the log-rank test. Only IgA positive outcome (≥ 8) proved to be statistically significant at $p=0.0548$, assuming a significance level at $\alpha=0.10$ (Figure 1). The other variables considered showed no significant impact on the survival rate, including gender ($p=0.9406$), positive IgG ($p=0.8577$), cardiomyopathy ($p=0.2558$).

A detailed analysis led to the joint consideration of both IgA and IgG levels. With four classes of positive/negative combinations, the differences in survival rate proved to be highly significant at $p=0.0040$ (Figure 2).

For multivariate analysis, all the variables considered were included in the initial Cox model. Then the final model was obtained using a backwards stepwise procedure (Figure 3).

It should be noted at this stage that all the patients with positive IgA and negative IgG died within 6 months of the surgery, whereas 1-year survival was noted in patients with negative IgA and IgG, and was estimated by the Kaplan-Meier method at 83.3%.

Discussion

Serological evidence is a useful instrument for the assessment of primary and chronic infections, both respiratory tract diseases [12] and cardiovascular diseases [13,14]. Serological tests are commonly regarded as inadequate in terms of facilitating localization of the infection processes and likely current activity of the pathogen. Culture-growing or PCR methods seem to be far more efficient [15,16,17] in establishing elevated, high-titered antibodies as markers of acute or chronic chlamydial infection in large epidemiological studies [4,5,18]. A review of laboratory methods for diagnosing Cp infections indicates that the expanded gold standard must include serology-based tests: microimmunofluorescence (MIF) or enzyme immunoassay (EIA) [19].

In chronic chlamydial infections, IgA antibodies seem to be a better marker than IgG antibodies, and are clearly

indicative of a failed immunological response against an intracellular bacterium. The prevalence of IgA antibodies after an acute infection is relatively short, due to their inherently shorter half-time, as opposed to IgG antibodies, which tend to disappear much less rapidly. Elevated and prolonged IgA titers are observed in chronic bacterial infections, and are generally considered to be indicative of a chronic infection process [20]. The prospective studies published to date have revealed that chronic Cp infection, as indicated by the incidence of elevated IgA titer, is an independent risk factor for the development of coronary heart disease [4,5] and asymptomatic carotid atherosclerosis [18]. In the present study, IgA level proved to be of better predictive value for post-OHT mortality than IgG antibodies.

Several mechanisms of chronic chlamydial infection account for the impact on OHT mortality. Cp may be introduced into the human system through infected peripheral blood monocytes [21], which are subsequently transformed into reservoirs for further infections. Cp harbored in monocytes remains metabolically active and participates in the maintenance of local immunological response [22]. It has previously been demonstrated that Cp has a direct impact on the activation of the proinflamatory and proliferative transcription factors, i.e. NF-κB and AP-1, in human vascular smooth muscle cells (VSMC) [23] and endothelium [24].

Chlamydial heat shock protein 60 (HSP60) induces tumor necrosis factor (TNF-α) and matrix metalloproteinase (MMP) expression [23], and antibodies to chlamydial HSP 60 mediate endothelial cytotoxicity [25]. This finding suggests that bacteria act on the initial defense mechanisms that mobilize humoral response to eliminate the microorganisms. This mobilization may, however, develop autoimmunological and inflammatory processes, manifested as the systemic activation of autoreactive T and B lymphocytes, followed by perivascular fibrosis, fibrinous occlusions, and thickening of the arterial walls [26].

In individuals with a failed immunodefense system (e.g. HIV-1 infected children), the incidence of Cp infection appears to correlate with the extent of immunosuppression [27]. In our population, pharmacologically maintained immunosuppression may be an important factor in desynchronizing humoral and cellular response, usually stimulated by a bacterial infection. In the case of Cp, which is an intracellular parasite, infection is usually of chronic character, and viable bacteria may persist within the tissue, to be prospectively cultured *in vitro*.

Immunosuppression facilitates the colonization of the human system by Cp and disturbs the balance between immunoresponse and cytotoxicity. In a vast majority of cases, chronic infection induced by Cp, usually of asymptomatic character, is hard to diagnose, and hence the elevated levels of IgG and IgA antibodies, or the presence of an ethiological factor within the system, constitute the only markers [4,5]. Given the current state of our knowledge in this respect, it would be reasonable to conjecture that chronic Cp infection does in fact have a profound impact on atherogenesis.

Our previous studies [28], conducted using DAKO antibodies labeled against Cp and a confocal microscope, attested to the presence of Cp bacteria in atherosclerotic lesions in the arteries and aortic walls of transplant recipients [29,30]; these findings have also been corroborated by other authors [31].

Our preliminary results require further confirmation, since the research was carried out on a relatively small group of patients. Long-term prognosis in patients after OHT may be influenced not only by the patient's clinical status, the professional expertise of the transplanting surgeons, and the effectiveness of immunosuppression therapy, but also by an adequate knowledge of Cp status.

Of the 24 study patients with ischemic cardiomyopathy, 12 (50%) died, as compared to 6 (35%) of the 17 patients with idiopathic cardiomyopathy ($p=0.0594$). This may have been due to chronic Cp infection, as the survival curves encountered in patients with positive IgA level seem to provide some grounds to prompt such a conclusion.

It should be noted at this juncture that the vast majority of deaths occurred within the first month after OHT, i.e. within the most critical period. No correlation was observed between the IgG level and the survival rate after OHT. Of the relative handful of reports dealing with this issue, only the article by Wittwer et al. [10] can be cited at this point. The authors pointed out that positive level of IgA against Cp affected the development of vasculopathy in the transplanted heart, which was not the case, however, with respect to elevated IgG.

Despite rather limited data on this subject, it may reasonably be assumed that the immunosuppressed state would render chronic infection a plausible agent in the etiology of CAV. Although chronic Cp infection may adversely affect the long-term prognosis after OHT, this hypothesis requires further research. In view of the inherently shorter half-life of IgA, as compared to IgG [32], monitoring is a matter of greater significance in detecting chronic Cp infection. Post-operative immunosuppresion may activate the inflammatory process, as observed by Fang et al. [33], who pointed out that the prevalence of Cp antibodies in patients after OHT was significantly higher (OR 13.7; 95%; CI 1.6 – 117.4), in comparison to controls.

The findings of the present study pertain only to a small number of patients, and so further research is required, preferably using the PCR technique to assay for Cp-DNA in leukocytes [30]. The acquisition of accurate data on the level of Cp antibodies in patients referred for OHT may well have potential for better long-term prognosis. The results yielded by ISAR-3 [34] seem to lend a certain plausibility to this hypothesis, as Roxithromycin treatment perceptibly improved the outcome after PCI. It may also be of some interest that Azithromycin treat-

ment in animals has been found to retard atherogenesis [35].

Although in the early post-operative period the therapeutic focus is predominantly on countering the transplant rejection reaction, it may be of tangible benefit to test for Cp infection. As evidenced by our research, chronic Cp infection may adversely affect the patient survival rate after OHT. Antibiotic treatment options should therefore be seriously considered, especially in patients with ischemic cardiomyopathy.

Admittedly, this issue is addressed only perfunctorily in the literature on the subject matter, thus warranting more complete investigations carried out on a much larger population sample, preferably through randomized, double-blind studies focused specifically on antibiotic treatment.

The incidence of Cp infection in heart transplant recipients may be an additional risk factor within the first year of OHT follow-up, especially with respect to patients with ischemic cardiomyopathy.

Elevated IgA titer may attest to the incidence of chronic Cp infection, accounting for perceptibly worse prognosis in patients after OHT. In the present study those patients with negative IgA and IgG antibodies against Cp showed a very high 1-year survival rate (83.3%).

More comprehensive investigations, however, should be carried out on a much larger population sample in order to extrapolate our present findings into a working assumption of much wider implications.

CONCLUSIONS

Chronic Cp infection, as expressed by high IgA level, seems to have an adverse impact on the survival rate within one-year after OHT. IgA titers against Cp in heart transplant recipients should therefore be assessed, as high values may be a predictive risk factor within the first post-operative year.

REFERENCES:

1. Kirsch M, Baufreton Ch, Nafte lDC et al:: Pretransplantation Risk Factor for Death after Heart Transplantation: The Henri Mondor Experience. J Heart Lung Transplant, 1998; 17: 268-77
2. Reece IJ, Painvin GA, Chandler BZ et al: Infection after Cardiac Transplantation: Treatment and Prognosis. Texas Heart Institute Journal, 1984; 11: 32-7
3. Grattan MT, Moreno-Cabral CE, Starnes VA et al: Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA, 1989; 261: 3561-6.
4. Saikku P, Mattila K, Nieminen MS et al: Serological evidence of an association of a novel Chalamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet, 1988; 2: 983-6
5. Saikku P, Leinonen M, Tenkanen L et al: Chronic Chlamydia pneumoniae infection as a risk for coronary heart disease in the Helsinki Heart Study. Ann Int Med, 1992; 116: 273-8
6. Danesh J, Collins R, Peto R: Chronic infection and coronary heart disease: is there a link ? Lancet, 1997; 350: 430-6
7. Scheller B, Markwirth T, Schiefer H, Hennen B: Evaluation of the role of Chlamydia pneumoniae in the pathogenesis of atherosclerosis - Review J Clin Basic Cardiol, 2000; 3: 155-66
8. Pieniążek P, Stępień E, Świat AZ et al: Detection of Chlamydia pneumoniae in coronary aortic lesions of atherosclerosis in explanted hearts - confocal microscopy analysis. Eur J Clin Invest, 1999; 29(Suppl 1): 271
9. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. Circulation, 2002; 105: 1135-43
10. Wittwer T, Pethig K, Heublein B at al: Impact of chronic infection with Chlamydia pneumoniae on incidence of cardiac allograft vasculopathy. Transplantation, 1999; 69: 1962-4
11. Mancini DM, Eisen H, Kussmaul W et al: Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. Circulation, 1991; 83: 778-86
12. Hahn D-L, Dodge R-W, Golubiatnikow R: Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA, 1991; 266: 225-30
13. Linnamäki E, Leininen M, Mattila K et al: Chlamydia pneumoniae - specific circulating immune complexes in patients with chronic coronary heart disease. Circulation, 1993; 87: 1130-4
14. Wesslen L, Pahlson C, Friman G et al: Myocarditis caused by Chlamydia pneumoniae (TWAR) and sudden unexpected death in a Swedish elite orienteer. Lancet, 1992; 340: 427-8
15. Maass M, Bartels C, Engel PM et al: Endovascular presence of Chlamydia pneumoniae DNA is common phenomenon in coronary artery disease. JACC, 1998; 31: 827-32
16. Ramirez JA and the Chlamydia pneumoniae/Atherosclerotic Study Group: Isolation of Chlamydia pneumoniae from the coronary atherosclerosis. Ann Intern Med, 1996; 125: 979-82
17. Molestina RE, Dean D, Miller RD et al: Characterization of a strain of Chlamydia pneumoniae isolated from a coronary atheroma by analysis of the omp1 gene and biological activity in human endothelial cells. Inf Immun, 1999; 66: 1370-6
18. Melnick S-L, Shahar E, Folsom A et al: Past infection by Chlamydia pneumoniae strain TWAR and asymptotic carotid atherosclerosis. Am J Med, 1993; 95: 499-54
19. Verkooyen RP, Hiep-Van Casteren SC a M, Mousavi Joulandan SA et al: Evaluation of PCR, culture and serology for diagnosis of Chlamydia pneumoniae respiratory infection. J Clin Microbiol, 1998; 36: 2301-7
20. Saikku P: Seroepidemiology in Chlamydia pneumoniae - atherosclerosis association. European Heart J, 2002; 23: 263-4
21. Gaydos CA, Summersgill JT, Sahney NN et al: Replication of chlamydia pneumoniae in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. Inf Immun, 1996; 64: 1614-20
22. Airenne S, Surcel HM, Alakarppa H et al: Chlamydia pneumoniae infection in human monocytes. Inf Immun, 1999: 67: 1445-9
23. Miller SA, Craig H, Selzman CH: Chlamydia pneumoniae activates nuclear factor kB and activator protein 1 in human vascular smooth muscle and induces cellular proliferation. J Surg Res, 2000; 90: 76-81
24. Molestina RE, Miller RD, Lentsch AB et al: Requirement for NF-?B in transcriptional activation of monocyte chemotactic protein 1 by chlamydia pneumoniae in human endothelial cells. Inf Immun, 2000; 68: 4282-8
25. Mayr M, Matzler B, Kiechl S et al: Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of Escherichia coli and Chlamydia pneumoniae: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. Circulation, 1999; 99: 1560-6
26. Bachmaier K, Neu N, La Maza LM et al: Chlamydia pneumoniae infections and heart disease linked through antigenic mimicry. Science, 1999; 283: 1335-9
27. Cosentini R, Esposito S, Blasi F et al: Incidence of Chlamydia pneumoniae infection in vertically HIV-1-infected children. Eur J Clin Microbiol Infect Dis, 1998; 17: 720-3
28. Pieniążek P, Karczewska E, Stępień E et al: Incidence of Chlamydia pneumoniae infection in patients with coronary artery disease subjected to angioplasty or by-pass surgery. Med Sci Monit, 2001; 7(5): 995-01

29. Taylor-Robinson D, Ong G, Thomas BJ et al: Chlamydia pneumoniae in vascular tissue from heart-transplant donors. The Lancet, 1998; 351: 1292

30. Boman J, Soderberg S, Forsberg J et al: High prevalence of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells in patient with cardiovascular disease and in middle-aged blood donors. J Infect Dis, 1998; 178: 274-7

31. Muhlestein JB, Hammond EH, Carlquist JF et al: Increased incidence of Chlamydia species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. J Am Coll Cardiol, 1996; 27: 1551-61

32. Wong YK, Gallagher PJ, Ward ME: Chlamydia pneumoniae and atherosclerosis. Heart, 1999; 81: 232-8

33. Fang JC, Kinlay S, Kundsin R, Ganz P: Chlamydia Pneumoniae infection is frequent but not associated with coronary arteriosclerosis in Cardiac Transplant recipients, Am J Cardiol, 1998; 82: 1479-83

34. Neuman FJ, Kastrati A, Miethke T et al: Treatment of Chlamydia pneumoniae infection with roxithromycin and effect on neointima proliferation after coronary stent placement (ISAR-3): a randomized, double-blind, placebo-controlled trial. The Lancet, 2001; 357: 2085-89

35. Muhlestein JB, Anderson JL, Hammond EH et al: Infection with Chlamydia pneumoniae accelerates the development of atherosclerosis and treatment with azithromycin prevents it in rabbit model. Circulation, 1998; 97: 633-6